





Longitudinal Time-series Analysis Of The Effects Of Long-duration Space Flight In Male And Female Astronauts Using A ^1H -nmr-based Metabolomics Approach



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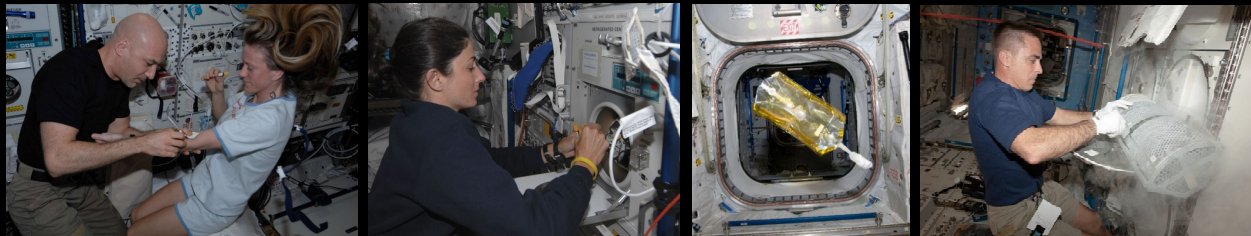
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Background

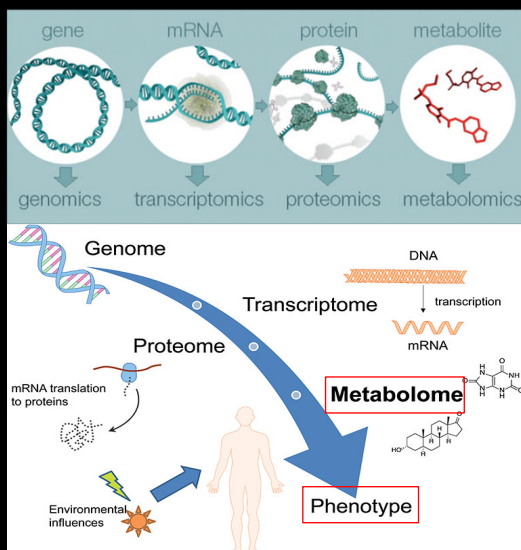
- Samples from Biochem Profile and Nutrition SMO studies
- Generate database of biochemical analyses from crews on ISS missions
- **Blood**/urine samples collected pre-, in-, and postflight
 - L-#: days before launch
 - FD-#: in-flight days
 - R+#: return to earth and recovery



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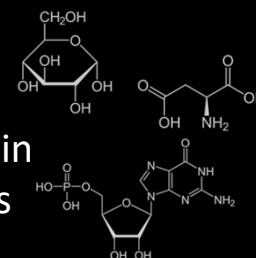
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Metabolomics



Metabolites: small molecules involved in metabolic processes

Metabolome: entire collection of metabolites in an organism



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Methods

¹H-Nuclear Magnetic Resonance Spectroscopy

- Sample Collection (♂n=40; ♀n=11)

- L-45, R+0, R+30

collected and allowed to clot → centrifuged → serum separated → frozen at -80°C

- L-10, FDs

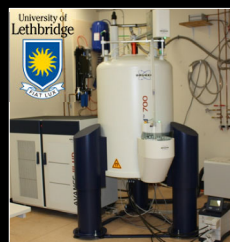
collected and allowed to clot → centrifuged → frozen at -80°C

L-10 + FDs

L-45 + R's

- Sample preparation for NMR

- Filtered macromolecules with Amicon ultra 3kDa filters
- Buffer, D₂O w/ TSP at 0.02709% w/v added
- Run on 700 MHz Bruker Avance III HD NMR spectrometer



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Analytics

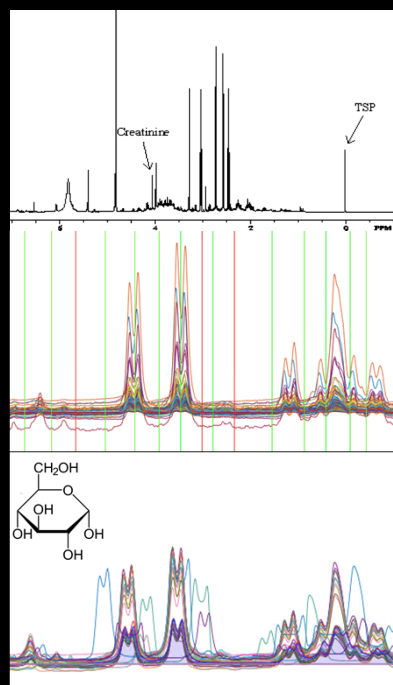
Spectral Processing and Data Reduction:

- Phase shift and baseline correction
- Dynamic Adaptive Binning
- U-matrix and statistics generation
- Metabolite identification
- Multivariate Bayesian Time Series Analysis
- Pathway Topology Analysis

Statistical Analysis:

- Multi-Variate Supervised: OPLS-DA, VIAVC
- Uni-Variate: paired t-test; $p < 0.05$

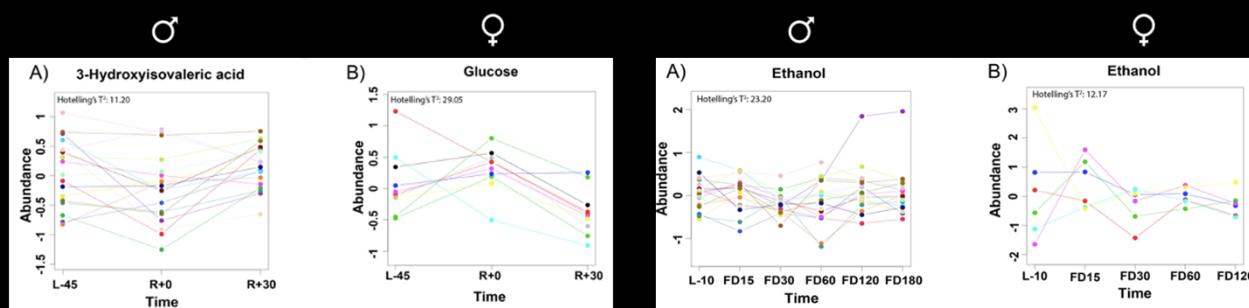
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MEBA

Blood serum is better suited for entry in and exit out of space



L-45 + R's

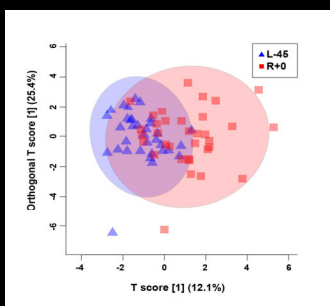
L-10 + FDs

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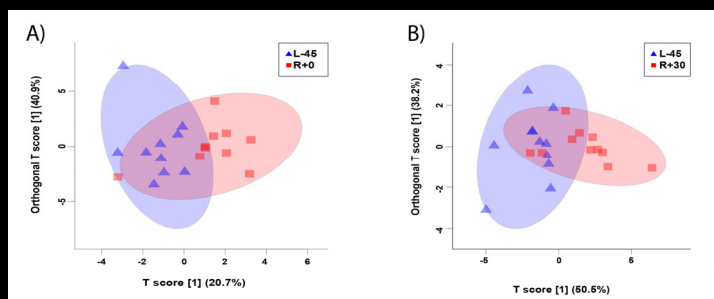
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Statistical Analysis

Females' metabolomes take longer to return to pre-flight levels



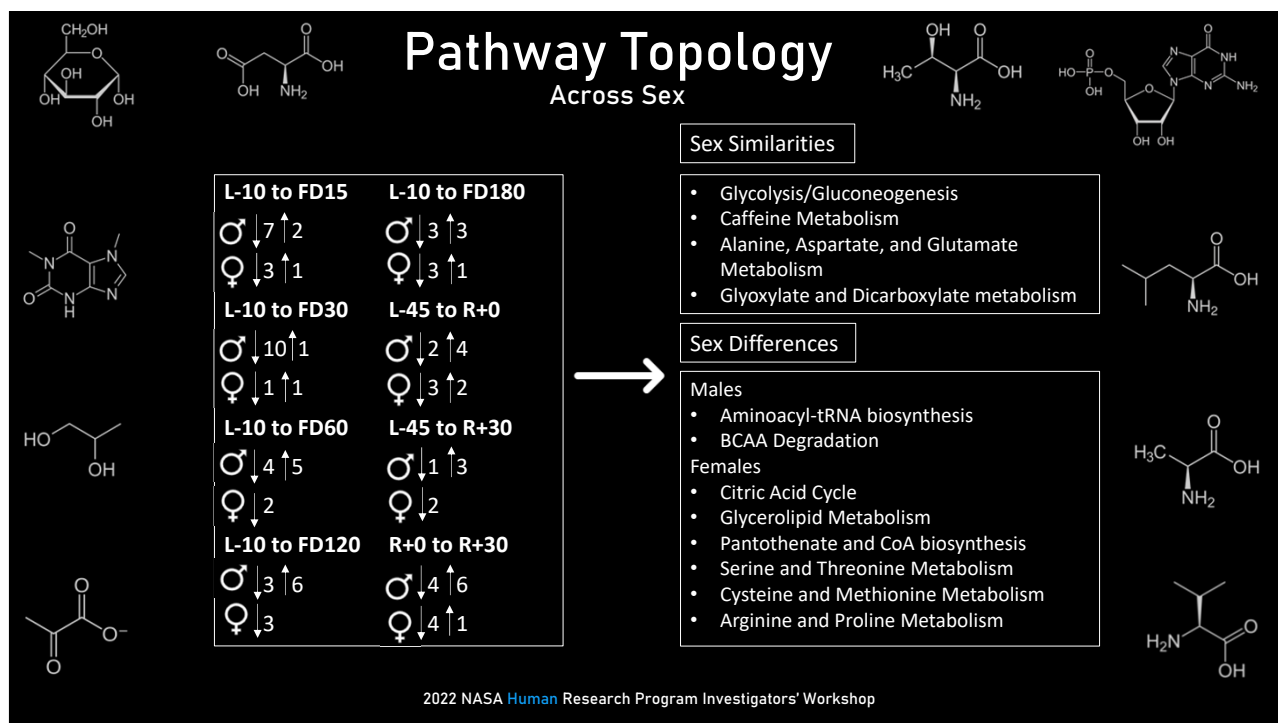
Male
Supervised OPLS-DA
VIAVC + paired t-test



Female
Supervised OPLS-DA models
VIAVC + paired t-test

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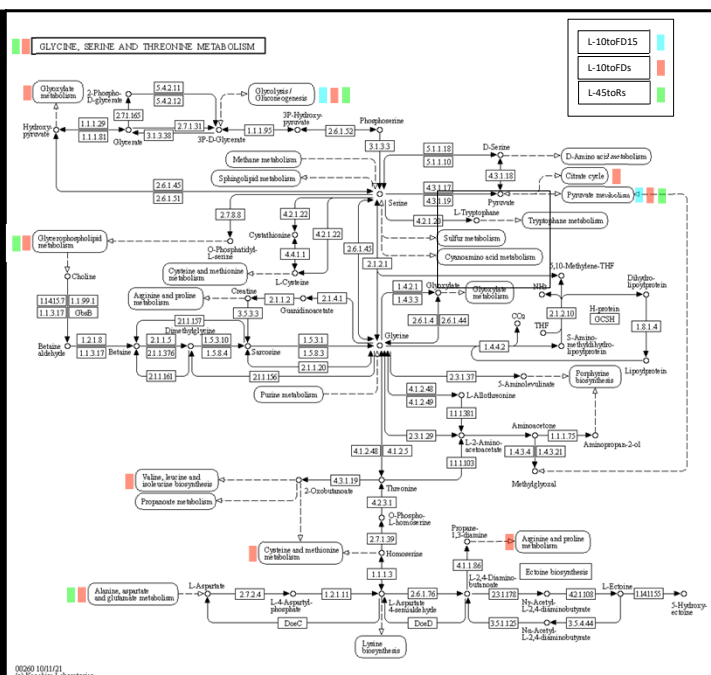
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Pathway Topology

Across Time

- Pre-launch rel. to entry into space: energy metabolism, immunity, & muscle and bone tissue maintenance
- Prolonged life in space: immunity, energy metabolism, macromolecule synthesis and maintenance, & muscle and bone tissue maintenance
- Pre-launch rel. to return and recovery: immune dysregulation upon return, other effects persist into recovery

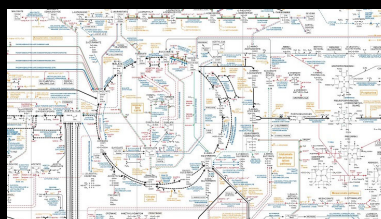
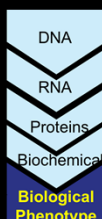
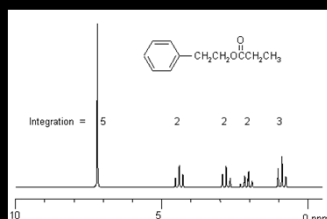


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Conclusions

- Perturbations overlapped across categories
- Overarching perturbations
- Sex differences
- ^1H -NMR spectroscopy and metabolomics utility

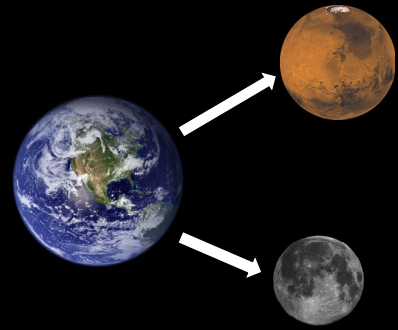


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Significance

- Countermeasure development
- Nutrition plans
- Contributes to knowledge gap
 - Metabolomics
 - ^1H -NMR as a viable technology
 - Sex differences
- Future human colonization of non-earthly celestial bodies
- Variety of (other) applications

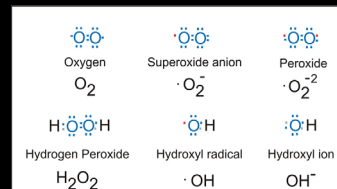


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Future Directions

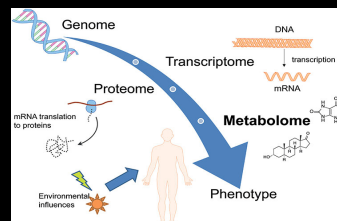
Correlation studies



Other technologies



Multi-'omics' approach*



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Acknowledgements

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